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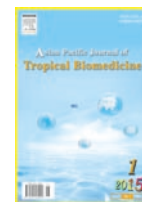


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In vitro vasodilatory effect of aqueous leaf extract of *Thymus serrulatus* on thoracic aorta of Guinea pigsBekesho Geleta^{1*}, Mebrahtu Eyasu², Selamu Kebamo³, Asfaw Debella¹, Eyasu Makonnen⁴, Abiy Abebe¹¹Directorate of Traditional and Modern Medicine Research, Ethiopian Public Health Institute, P.O. Box 1242, Addis Ababa, Ethiopia²St. Paul's Hospital Millennium Medical College, Department of Pharmacology, P.O. Box 1271, Addis Ababa, Ethiopia³Wollega University, Department of Pharmacology, P.O. Box 395, Nekemte, Ethiopia⁴Addis Ababa University, College of Health Sciences, School of Medicine, Department of Pharmacology, P.O. Box 9080, Addis Ababa, Ethiopia

PEER REVIEW

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Hawassa, Ethiopia.**Comments**

Over all the paper is informative. All the sections are written well and in proper flow, with details of exclusion and inclusion criteria in materials and methods to avoid flaws in findings. Its my pleasure to go through this paper.

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ABSTRACT

Objective: To investigate the vasodilatory effect of *Thymus serrulatus* (*T. serrulatus*) aqueous leaf extract on KCl (high K⁺, 80 mmol/L) induced precontracted isolated thoracic aorta rings on guinea pigs and the role of aorta endothelium on this action.

Methods: Guinea pig thoracic aorta was removed and placed in an organ bath containing Krebs-Henseleit solution and aorta contractions were recorded isometrically.

Results: The results revealed that *T. serrulatus* aqueous leaf extract (0.5-5 mg/mL) significantly ($P < 0.001$) reduced KCl-induced contractions of guinea pig thoracic aorta in both intact ($n=5$) and denuded ($n=5$) endothelium in a concentration dependent manner, and the vasodilatory effect of the extract on intact endothelium was significantly ($P < 0.05$) higher than that on denuded endothelium. Glibenclamide (10 $\mu\text{mol/L}$) significantly ($P < 0.001$) increased the vasodilatory effect of extract in intact endothelium as compared to methylene blue (10 $\mu\text{mol/L}$), atropine (10 $\mu\text{mol/L}$) and indomethacin (10 $\mu\text{mol/L}$). The effect was more obvious on intact than that on denuded endothelium.

Conclusions: The present findings demonstrate that *T. serrulatus* aqueous leaf extract has vasodilatory activity which might result in antihypertensive effect and its vasodilatory effect is endothelium-dependent. This might support the traditional claim of the plant in hypertensive.

KEYWORDS

Thymus serrulatus, Guinea pig, Aqueous extract, Endothelium, Organ bath, Vasodilatory effect**1. Introduction**

Hypertension, also known as high or raised blood pressure, is a chronic disorder characterized by a persistently elevated blood pressure exceeding 140/90 mmHg or greater^[1-3]. It has been named the "silent killer," as it is asymptomatic and the major contributor or risk factor to cardiovascular morbidity and mortality^[4]. Hypertension is a leading cause of cardio vascular disorder such as myocardial infarction and stroke worldwide^[5,6]. Various herbal preparations have been used and claimed to have benefit for hypertension in the folk medicine such as *Thymus serrulatus* Hochst. ex Benth (*T. serrulatus*).

Ethiopian traditional medicine is composed of a number of specific skills, such as, use of plants, animal products and minerals as well as magic and superstition. The main body, however, is based on the use of ethnobotany^[7]. *Thymus*, an aromatic plant belonging to the Lamiaceae family^[8,9], has been reported to be found in different parts of Ethiopia. The two species, *Thymus schimperi* Ronniger and *T. serrulatus*, are locally known as Tosign in Amharic, and both are endemic to the Ethiopian highlands. They grow on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes. Both species are perennial herbs, woody at the base and are 5-40 cm high^[8].

T. serrulatus is one of the most traditionally used herbal medicines

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that is also used as a spice and food preservative. Moreover, it is also used for its antioxidant effect in food, aroma industries and as culinary ingredient. The ethanol extract or essential oil of thyme has a significant rate of antifungal and antimicrobial activities with strong lipid peroxidation inhibitory and high hydroxyl radical scavenging activities[10].

T. serrulatus leaves are traditionally used to make herbal tea and juice for the treatment of hypertension and renal diseases[11] as well as diuretic, anthelmintic, antispasmodic, carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative, tonic, anti-inflammatory and libido enhancer[9,12,13]. To further substantiate the traditional claimed medicinal use of the plant for its hypertension, the present study was carried out on the vasodilatory effect of *T. serrulatus* aqueous leaf extract on potassium chloride induced precontracted guinea pig thoracic aorta.

2. Materials and methods

2.1. Plant collection and authentication

The fresh leaves of *T. serrulatus* were collected from North-west Ethiopia around Debre Sina town, 180 km far from Addis Ababa in April 2013. The plant material was authenticated by a botanist in the Directorate of Traditional and Modern Drug Research, Ethiopian Public Health Institute.

2.2. Preparation of the crude extract

Fresh leaves of *T. serrulatus* were dried at room temperature and crashed with mortar and pestle. A total of 350 g of the powdered leaves were soaked in hot distilled water while shaken using electric shaker for 1 h. The extract was first filtered with gauze then with Whatman filter paper No. 1, 15 cm size, and freeze dried or lyophilized (Labconco, USA) overnight to give amorphous powder of total yield 9.3% (w/w) which was collected in vial and kept in a refrigerator until used for the experiment.

2.3. Animals

Guinea pigs (*Cavia porcellus*), of male sex weighing between 350-400 g, were obtained from the animal house of the Ethiopian Public Health Institute. The guinea pigs were housed in a room with constant temperature and at 12 h of light/dark cycle. The animals were fed and given water *ad libitum* until they were used for this experiment. The animals were generally used according to guideline for use and care of animals[14].

2.4. In vitro experiment of thoracic aorta

The *in vitro* vasodilatory study was conducted on thoracic aortas of guinea pig according to the methods described by Gilani *et al*[15]. The guinea pigs were killed by gentle cervical dislocation, and the thoracic cavity was opened, then the aorta was identified. The descending thoracic aorta was then immediately removed after being freed from connective tissues. Each aorta was cut spirally to make strips of about 3 mm wide and 4 cm long. The strips were immediately mounted in an organ bath containing 20 mL Krebs-Henseleit physiological solution (at room temperature), which was suspended between two L-shaped stainless steel hooks. The ionic compositions of the Krebs-Henseleit solution were as follows (with mmol/L): Na⁺ 137.4, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, HCO₃⁻ 15.5, H₂PO₄ 1.2, Cl⁻ 134 and glucose 11.5. The solution was maintained at 37 °C, continuously aerated with carbogen (95% O₂+5% CO₂ gas mixture) at a pH of 7.4. Experiments were performed in preparations with intact and denuded (removed) endothelium. A

resting tension of 1-1.6 g was applied to the tissue and allowed to stabilize for about 1-2 h before adding any chemical [methylene blue (10 μmol/L), atropine (10 μmol/L), indomethacin (10 μmol/L) or glibenclamide (10 μmol/L)] during which it was washed every 15 min. After stabilization, the aorta was contracted with KCl (high K⁺, bath concentration of 80 mmol/L). Once a contraction plateau was achieved, increasing concentrations of the aqueous leaf extract of *T. serrulatus* were cumulatively added every 3 min, and tension changes of the tissue were recorded. The effect of extract on resting tension was tested with isometric sensors and traced using a polygraph (Model 7E, Grass Inc., USA).

2.5. Drugs and reagents

Potassium chloride (Chadwell Essex, UK), atropine and methylene blue (Sigma Aldrich, Germany), indomethacin, glibenclamide (Remedica, Cyprus) and Krebs-Henseleit solution were used in the study. All the drugs and reagents used were with the required standard and analytical grade.

2.6. Statistical analysis

Results were expressed as mean±SEM and were subjected to biostatistical interpretation by SPSS windows version 20 statistical packages all the way through a One-way and Two-way ANOVA followed by *post-hoc* test (Tukey's test) for multiple comparisons. A *P*<0.05 was considered as level of significance.

3. Results

3.1. Effect of *T. serrulatus* aqueous leaf extract on KCl induced precontracted aorta and the role of endothelium

Sequential administrations of relatively low to high concentrations of *T. serrulatus* aqueous leaf extract (0.5-5 mg/mL) to the organ bath fluid significantly (*P*<0.001, in all cases) attenuated the force of contractions on thoracic aorta of guinea pigs, which were provoked by KCl in concentration dependent manner both in intact (*n*=5) and denuded (*n*=5) endothelium (Figure 1).

The percent of aorta relaxation of the *T. serrulatus* aqueous leaf extract showed a significant (*P*<0.05) difference between intact and denuded endothelium of the guinea pigs thoracic aorta with a statistical mean of (43.10±0.52)% and (35.60±0.52)% respectively.

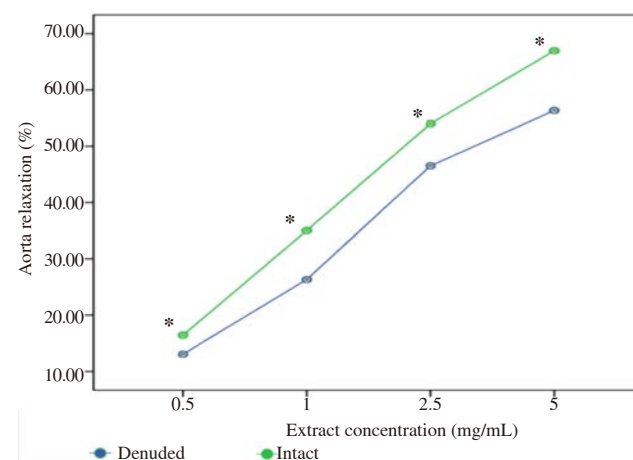


Figure 1. The vasodilatory effect of *T. serrulatus* aqueous leaf extract on intact (*n*=5) and denuded (*n*=5) endothelium of guinea pigs thoracic aorta precontracted by KCl (80 mmol/L).

*: Significant (*P*<0.05) difference between the vasodilatory effect of *T. serrulatus* aqueous leaf extract on intact and denuded endothelium at the same concentrations. Two-way ANOVA was used, *P*<0.001.

3.2. Effects of glibenclamide and various vasoconstrictors on endothelium-dependent vasodilatory effect of *T. serrulatus* aqueous leaf extract

The presentation in Figure 2 illustrated that the *T. serrulatus* aqueous leaf extract on intact ($n=5$) endothelium guinea pigs thoracic aorta showed a significant ($P<0.001$) synergistic effect in glibenclamide induced dilation. However, the percent of aorta dilation with the extract in intact endothelium used together with vasoconstrictor, such as atropine, methylene blue or indomethacin, did not show any significant change ($P>0.05$) compared to that without vasoconstrictors.

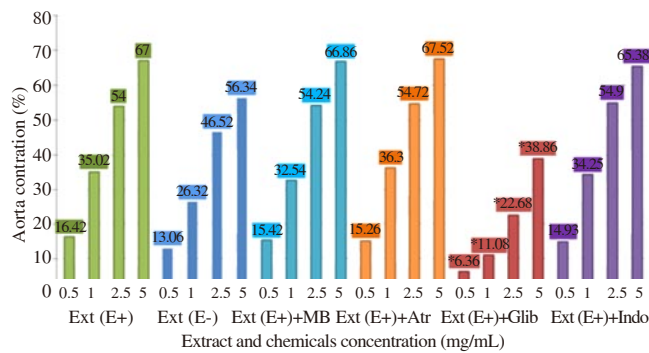


Figure 2. Effects of glibenclamide and various vasoconstrictors on vasodilatory effect of *T. serrulatus* aqueous leaf extract on KCl induced precontractions of guinea pigs thoracic aorta.

Ext: Extract; E+: With intact endothelium; E-: With denuded endothelium; MB: Methylene blue; Atr: Atropine; Glib: Glibenclamide; Indo: Indomethacin. Asterisk indicates aorta dilation of Ext (E+)+glibenclamide was significant ($P<0.001$) compared to the rest concentrations.

4. Discussion

The KCl-induced contractions on the guinea pigs isolated thoracic aorta (with intact and denuded endothelium) were significantly ($P<0.001$, in all cases) attenuated by *T. serrulatus* aqueous leaf extract (0.5-5 mg/mL) in a concentration dependent manner, and the vasodilatory effect of the extract was significantly higher ($P<0.05$) on intact endothelium than denuded endothelium rings at all concentration used. Therefore, the results of the present study indicate that *T. serrulatus* aqueous leaf extract possesses endothelium-dependent vasodilatory effect. This finding is in agreement with other studies done on vasodilatory effect of leaves of *Sesamum radiatum* Schum. & Thonn.[16], *Bridelia ferruginea*[17], *Cistus ladaniferus*[18], *Globularia alypum*[19], *Salvia miltiorrhizae*[20], *Pueraria lobata*[20], *Ficus deltoidea*[21] and *Loranthus ferrugineus*[22], on same species.

T. serrulatus aqueous leaf extract significantly ($P<0.001$) decreased the contractions provoked by KCl on the guinea pigs isolated thoracic aorta intact endothelium, while concurrently used with glibenclamide, a standard drug as compared with the intact endothelium rings alone. However, simultaneous use of vasoconstrictors, such as methylene blue, atropine and indomethacin, did not increase the vasodilatory effect of the extract significantly ($P>0.05$) on the previous contractions made by KCl. This could explain why the mechanism of the vasodilatory effect of *Thymus* is similar to that of glibenclamide. Many evidences show that exposure of intact endothelium rings to glibenclamide raises the tissue content of cGMP by approximately 2-fold, which not only mediates

nitricoxide-dependent relaxation, but also inhibits contractile response of some vascular tissues to a number of constrictors, such as high K^+ , phenylephrine and endothelin-I. This suggests that glibenclamide might have multiple sites of action[23,24].

The present findings demonstrate the vasodilatory effect of *T. serrulatus* aqueous leaf extract which supporting the traditional claim for its antihypertensive activity. Further investigations, however, are needed to come up with the exact mechanism of its vasodilatory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Hypertension is one of the current emerging community health problem, which is very common affecting one in four individuals and it is associated with the cardiovascular disorder, diabetes mellitus, dyslipidemia which is considered as metabolic syndrome. Since there are various etiological factors associated with hypertension, it is very difficult to predict which one is the most common cause of hypertension. The current study is based on the association of hyperuricemic with hypertension. The study tries to exemplify the reasonable mechanism(s) through which higher uric acid levels inadvertently affect the blood pressure by controlling the confounding factors.

Research frontiers

The present study was performed to investigate the vasodilatory effect of *T. serrulatus* aqueous leaf extract on KCl (high K^+ , 80 mmol/L) induced precontracted guinea pigs' isolated thoracic aorta rings and the role of aorta endothelium on this action, and it demonstrates the vasodilatory activity which might result to antihypertensive effect and its vasodilatory effect is endothelium-dependent. This is the first study and it has immense importance in treatment of hypertension.

Related reports

No such studies have been reported earlier, but this study is first of its kind.

Innovations and breakthroughs

It is for the first time in the extract of *T. serrulatus* aqueous leaf extract that has been exploited as a vasodilator and thus it can be published.

Applications

Aqueous extract of *T. serrulatus* leaf can be further researched on its applied medicine in other areas of diseases apart from

hypertension.

Peer review

Over all the paper is informative. All the sections are written well and in proper flow, with details of exclusion and inclusion criteria in materials and methods to avoid flaws in findings. Its my pleasure to go through this paper.

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